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User's Manual and Instructions

ELISA Kit for IgM Antibody to Hepatitis A Virus

Catalog No.: KO31008096

[NAME AND INTENDED USE]
ELISA Kit for IgM Antibody to Hepatitis A Virus is an *in vitro* enzyme immunoassay for the detection of HAV-IgM in human serum or plasma.

[PRINCIPLE]
This kit uses capture ELISA method to detect anti-HAV IgM. The purified rabbit anti human IgM monoclonal antibody (Anti-μ-chain) is coated on the solid phase of multi-wells. Serum sample, Horseradish peroxidase labeled HAVAg are added to coated wells. After incubation, if HAV-IgM is present in the sample, a complex of Anti-μ-chain-HAV-IgM-HAVAg labeled with HRP will form. Wash wells to remove other unbounded serum components, incubate with substrate (TMB) to form a colored product, and measure the absorbance at 450 nm to indicate the presence or absence of HAV-IgM in the sample. The test is special, sensitive, reproducible and easy to operate.

[MATERIALS PROVIDED]

1. Anti-μ-chain Coated Microwell Plate	1 block (96wells)
2. Enzyme Conjugant (HAVAg-HRP)	1 bottle (12ml)
3. Negative Control Serum	1 vial (1ml)
4. Positive Control Serum	1 vial (1ml)
5. Wash Buffer (1:20 dilution prior to use)	1 bottle (50ml)
6. Substrate A	1 bottle (6ml)
7. Substrate B	1 bottle (6ml)
8. Stop Solution	1 bottle (6ml)
9. Plastic Bag	1 bag
10. Seal Paper	3 pieces
11. Manual	1 each

[SAMPLE COLLECTION AND PRESERVATION]
Blood serum samples are routinely prepared form vein. Blood plasma sample are routinely prepared with routine amount of anticoagulant such as heparin or sodium citrate. Sample can be stored at 4°C if tested within five days. Sample can be stored at -20°C at least for 3 months. Avoid hemolysis and repetitive freeze and thaw of samples. Samples with cloud or precipitation should be centrifugated or filtered before test. Prevent serum from bacteria contamination during collection and storage.

[TEST PROCEDURE]
1. Bring *** ELISA Kit for IgM Antibody to Hepatitis A Virus *** (all reagents), and samples to room temperature before use (approximately 30 minutes).
2. Dilute concentrated wash buffer 1:20 with ddH_2O
3. For each test, set one blank, two positive and three negative controls. Add 50 μl positive and negative control serum into positive and negative control wells respectively without sample diluent.
4. Add 50 μl test serum into test wells.
5. Cover wells with seal paper, incubate for 30 minutes at 37°C.
6. Discard the liquid in all wells and fill the wells with wash solution (300μl per well). Lay aside for 15 seconds, discard the liquid in all wells and fill the wells with wash solution. Repeat 5 times and dry wells after last wash.
7. Add 100 μl Enzyme Conjugant in each well except the blank well.
8. Cover wells with seal paper, incubate for 30 minutes at 37°C.
9. Wash 5 times the same as step 6.
10. Add 50 μl substrate A and B respectively to each well, mix gently, protected from light and lay aside for 15 minutes at 37°C.
11. Add one drop of stop solution (50 ul) into each well to stop the reaction, including blank well.
12. Measure the absorbance at 450 nm against the blank, or measure the absorbance at 450 nm/630-690 nm.

[INTERPRETATION OF RESULTS]
Colorimetric Method
Cut Off Value calculation:
COV = 0.07 + the average OD of negative controls
Positive OD_{450} of sample COV
Negative OD_{450} of sample COV
Notes If the absorbance of negative controls is below 0.05, calculate it as 0.05. If the absorbance of negative controls is above 0.05, calculate it as its original value.

[PRECAUTIONS]
1. The samples should be fresh, avoid hemolysis, bacteria growing, and repetitive freeze and thaw.
2. Do not interchange reagents between kit lots. The seal paper can't be used repeatedly.
3. Mix reagents well before use. If crystal form in certain reagents, such as wash buffer, it can be used without problems after warm up and mix well.
4. Follow instruction exactly during assay, especially in temperature and time for reactions. All pipetting devices should be used with care and calibrated regularly following the manufacturer's instructions.
5. Put the remained reagents to the sealed pouch, and return to 2~8°C in time.
6. To prevent cross-contamination, wear gloves and working suits throughout the procedure, and execute the disinfection and isolation regulations strictly. Dispose of all samples and materials used to perform the test. The 5.0g/L liquid sodium hypochlorite solution or 121°C high pressure steam may be used to disinfect samples and materials before disposal (The positive control serum in the kit has been inactivated already)

[PACKAGE SIZE]
96 tests/Kit

[STORAGE AND STABILITY]
Store the kit at 2~8°C.

[EXPIRATION]
The shelf life is 12 months from the receiving date.

This Kit is for Research Use Only